

**CONSTRUCTION OF A DEFENSIVE TRASH PACKET FROM
SYCAMORE LEAF TRICHOMES BY A CHRYSOPID LARVA
(NEUROPTERA: CHRYSOPIDAE)**

THOMAS EISNER, JAMES E. CARREL, EILEEN VAN TASSELL,
E. RICHARD HOEBEKE, AND MARIA EISNER

(TE, ME) Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, U.S.A. (e-mail: te14@cornell.edu; mle3@cornell.edu); (JEC) Division of Biological Sciences, University of Missouri, Columbia, MO 65211, U.S.A. (e-mail: carrelj@missouri.edu); (EVT) Department of Entomology, Michigan State University, East Lansing, MI 48824, U.S.A. (e-mail: vantasse@pilot.msu.edu); (ERH) Department of Entomology, Cornell University, Ithaca, NY 14853, U.S.A. (e-mail: erh2@cornell.edu)

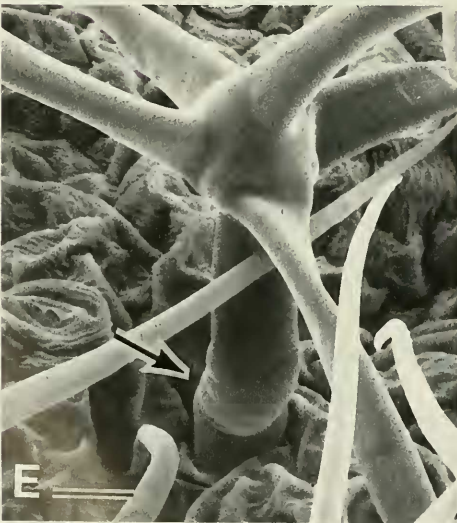
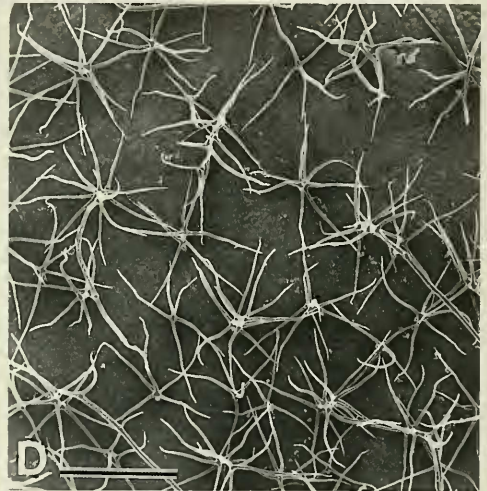
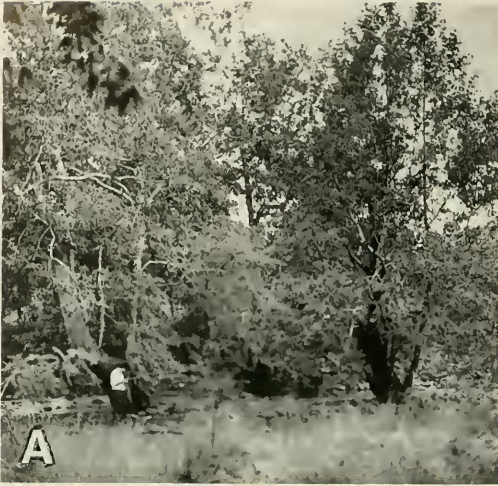
Abstract.—The behavior of a chrysopid larva from Arizona is described, identified as *Ceraeochrysa lineaticornis* (Fitch), which constructs its trash packet from the trichomes of sycamore leaves (*Platanus wrightii* S. Watson). The trichomes are particularly dense on the underside of the leaves. To fashion the packet, the larva uses trichomes stuck loosely to the leaf, and others that it plucks from the leaf surface. The packet on the mature larva weighs on average 2.2 mg, an equivalent of approximately two leaf undersides worth of trichomes. Evidence is presented indicating that the packet provides the larva with protection against predation. It is argued that the trichomes are defensive in the sycamore tree itself, and that the chrysopid provides yet another example of an insect that benefits from utilization of a plant defense. Interestingly, the chrysopid benefits the sycamore tree itself. Although it usurps the tree's defense, it aids the tree by preying on a specialist herbivore, the tingid, *Corythucha confraterna* Gibson, which feeds on the sycamore tree unbothered by the trichomes.

Key Words: animal defense, Neuroptera, Chrysopidae, *Ceraeochrysa lineaticornis*, plant defense, trichome, insect-plant interaction

Many chrysopid larvae have the habit of collecting exogenous materials and placing them on their backs, forming so-called trash packets that they retain throughout larval life (Smith 1922, Canard et al. 1984). The materials they use to fashion the packets are variable and may consist of vegetable matter, arthropod remains, insect waxes, or general debris (Smith 1922, Slocum and Lawrey 1976, New 1969, Eisner et al. 1978, Canard et al. 1984). Existing evidence indicates the packets act as physical shields that provide the larvae with protec-

tion against insectan predators (New 1969, Principi 1946, Eisner et al. 1978). Not all chrysopid larvae are trash carriers, but those that are, are obligatorily so, and the priority that larvae give to forming trash packets has a significant genetic component (Milbrath et al. 1993, Tauber et al. 1995).

Here we describe the behavior of a chrysopid larva that constructs its packet from trichomes that it takes from the leaves of sycamore trees (*Platanus wrightii* S. Watson) in Arizona. We present data on the feeding habits of the larva, as well as on



how the packet is constructed and used in defense. Our observations were made in the hamlet of Portal, Cochise County, Arizona, in 1967 (September) and 1982 (August). We refrained from publishing earlier because we had failed to obtain a definitive identification of the chrysopid. This problem has now been remedied, thanks to the courtesy of Catherine and Maurice Tauber, who have informed us that the chrysopid is *Ceraeochrysa lineaticornis* (Fitch) (voucher specimens have been deposited in the Cornell University Insect Collection).

MATERIALS AND METHODS

Field site.—We first noted the larvae on a group of sycamore trees growing on the banks of Cave Creek, in Portal itself, on the grounds of the Cave Creek Ranch, where we were in residence (Fig. 1A). We subsequently found them also on sycamore trees near Cave Creek, up to several miles upstream from the original location. The larvae were readily spotted by their white trash packet, which rendered them extremely conspicuous against the green color of the sycamore leaves on which they were found (Fig. 2A). As they scurried about, they resembled tiny ambulatory cotton wads. Careful scrutiny of the visually accessible lower branches of the trees usually revealed presence of many larvae per tree.

Maintenance of larvae.—Larvae were maintained on freshly clipped sycamore leaves in plastic containers of various sizes, including Petri dishes. In the field, larvae had been found on repeated occasions feeding on a tingid, *Corythucha confraterna* Gibson, whose colonies were of common occurrence on the sycamore leaves. Captive chrysopids were therefore always provided with some leaves that were tingid-infested.

They took readily to the tingids, and appeared to feed on the nymphs only. Maintained on this diet many of the larvae went on to pupate and develop into adults. As is typical for trash-carrying chrysopids, larvae retained the trash packet as an outer covering of their cocoon when they pupated. The cocoons therefore have the same appearance as the larvae, except that they are non-ambulatory. In the field we found several cocoons on the trunks of sycamore trees. Most larvae that we used in our experiments were probably in their last instar.

Electronmicroscopy.—For examination with the scanning electronmicroscope, specimens (chrysopid larvae, pieces of sycamore leaf) were preserved in the field in 70% ethanol, then critical point dried and gold coated in the laboratory.

Predation tests.—Of the predators used, the reduviid, identified as *Pselliopus latispina* Hussey, is doubtless a natural enemy of the chrysopid. It occurred commonly on the leaves and branches of the sycamore trees themselves, and was once actually observed feeding on a *C. lineaticornis* in the field.

The ant used in predation tests, *Pogonomyrmex barbatus* (Smith), may not itself be a primary enemy of the chrysopid, but there can be little question that ants as such figure among the larva's natural predators. We routinely observed ants foraging singly on the leaves, branches and trunks of the sycamore trees.

Statistics.—Numerical averages are given as mean \pm S.E.

RESULTS

The trichomes.—Both surfaces of the sycamore leaves bear trichomes, but the structures are much more densely distrib-

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Fig. 1. A, Stand of sycamore trees on which the chrysopid larva was discovered (Portal, AZ). B, Close-up of underside of sycamore leaf showing trichomes. C, Underside of sycamore leaf from which the trichomes have been removed; the detached trichomes form the wad seen in the ampule. D, Enlarged view of leaf underside (scanning electronmicrograph). E, Base of a trichome showing the hinge (arrow). F, Base of a trichome that has been torn off at the level of the hinge. Reference bars: D = 0.5 mm; E = 20 μ m.

uted on the underside (Figs. 1B, D). Individually the trichomes are usually multiply branched, and they are flexibly hinged near their base, where there is a break in the core of the shaft and the wall is reduced to a thin-walled tube (Figs. 1E, F). If one pulls on individual trichomes with forceps, they tend to detach at the level of these hinges. They evidently detach spontaneously in large numbers from the leaf surface, as evidenced by the fact that the underside of sycamore leaves usually has a loose coating of detached trichomes. Microscopic examination verified clearly that these loose trichomes had broken off at the level of the hinges.

Packet construction.—Removal of the trash packets from the larvae could be effected easily by teasing away the trichomes with forceps. Eight larvae that were thus denuded and re-released into their leaf-containing enclosures, commenced reloading almost at once. In typical chrysopid fashion (New 1969, Principi 1940, Smith 1922), using their jaws as a two-pronged fork, they scooped up load upon load of trichomes and placed these upon their backs (Figs. 2C–F). Most often they procured trichomes from the clusters of available loose ones, but they also pried many off with their jaws. It seemed clear, moreover, that they were programmed to utilize sycamore trichomes only. If released in a Petri dish with general particulate debris they tended to ignore such matter, but if then offered a sycamore leaf, they usually returned promptly to the task of reloading. Like trash-carrying chrysopids generally (Canard et al. 1984), the larvae are equipped with a set of special hooked bristles that seem to serve specifically for retention of the packet. The bristles project upward in rows from the back, and there are others that project outward in clusters from a series of lateral protuberances (Figs. 4A–C).

Packet construction appeared to proceed in accord to a fixed protocol. The first pluckings were always applied to the posterior third of the body. To gain access to

the site, the larvae arched that region upward and forward every time that the head flexed backward to deliver a load. Later pluckings, delivered to the more accessible anterior regions of the back, were applied without simultaneous postural adjustments of the body.

A visual demonstration of the sequence of trichome delivery during packet construction was obtained by giving denuded larvae access to trichomes of different colors. Packets were removed from a series of larvae and were then stained either in red (with acid fuchsin), in black (with chlorazol black), or kept unstained. Denuded larvae that were then given access to teased apart samples of these packets, in the sequence of black (for 30 min), to unstained (for 20 min), and to red (for 60 min), constructed packets in which the trichomes were laid out in three colored bands, in the sequence red, white, and black from fore to aft (Fig. 2B).

Package repair.—Use of stained trichomes also yielded visual evidence of the precision with which the larvae are able to repair damage to their package. Four larvae, bearing natural unstained packets, were treated as follows:

- (1) Trichomes removed from center of packet, leaving the larva with a ring-shaped shield.
- (2) Posterior half of packet removed.
- (3) Left half of packet removed.
- (4) No trichomes removed (control).

The larvae were then confined with a supply of black trichomes, and checked for condition of their packets after 24 hours. The results were as follows:

- (1) Center of ring filled with unstained trichomes. Periphery of packet loosely laden with black trichomes. Larva had evidently repaired the hole in the packet with trichomes from the periphery of the packet. New trichomes had been added to the periphery to replace those used in the repair of the hole.

- (2) Posterior half of packet repaired, but

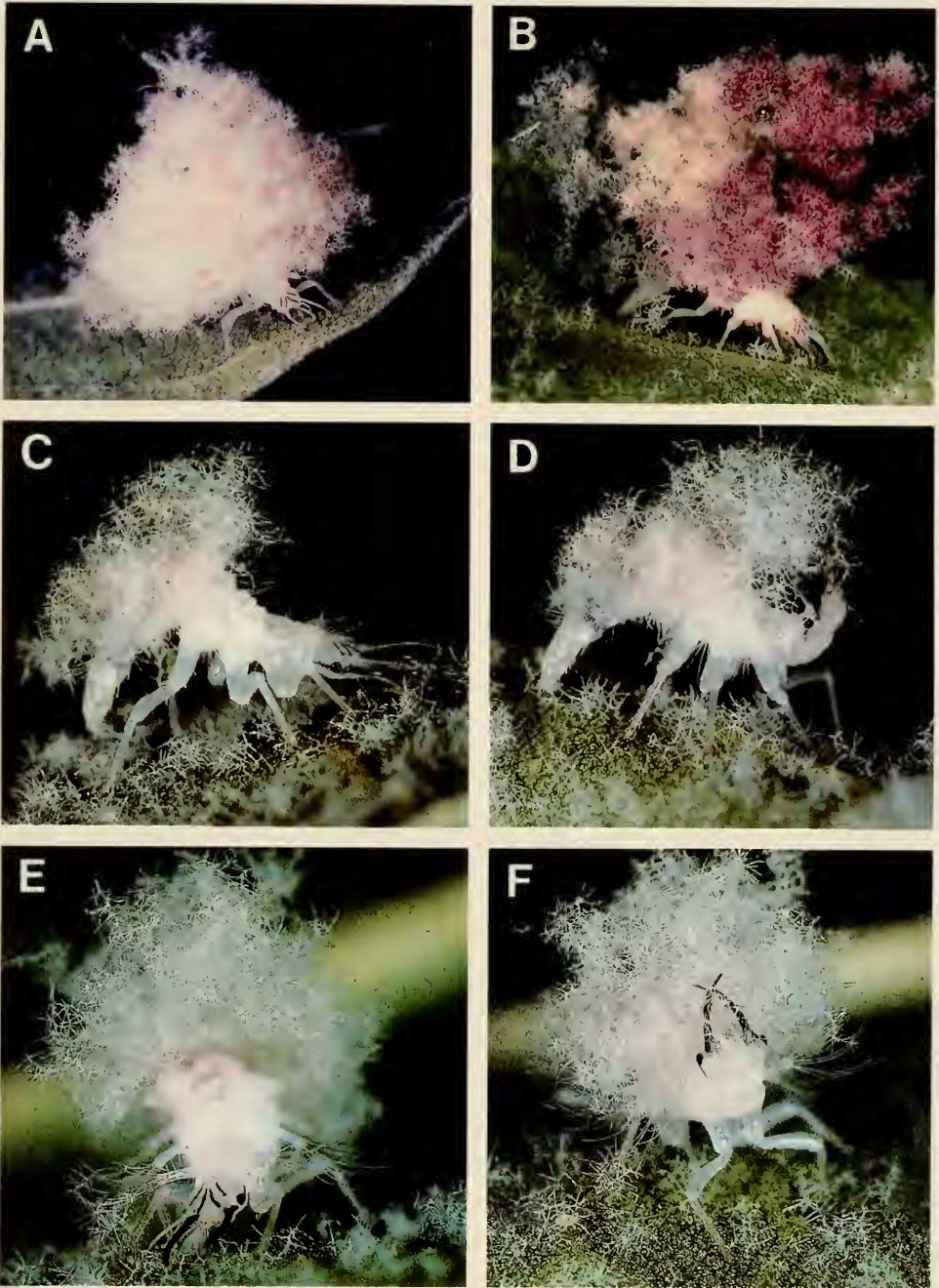


Fig. 2. A. Full-grown larva. B. Larva that has formed a packet from pre-stained trichomes (see text). C, D. Larva, in lateral view, in the process of building its packet. In C it is scooping up trichomes with the mouthparts; in D it is adding the trichomes to the packet. E, F. Same as preceding pair, but in frontal view.

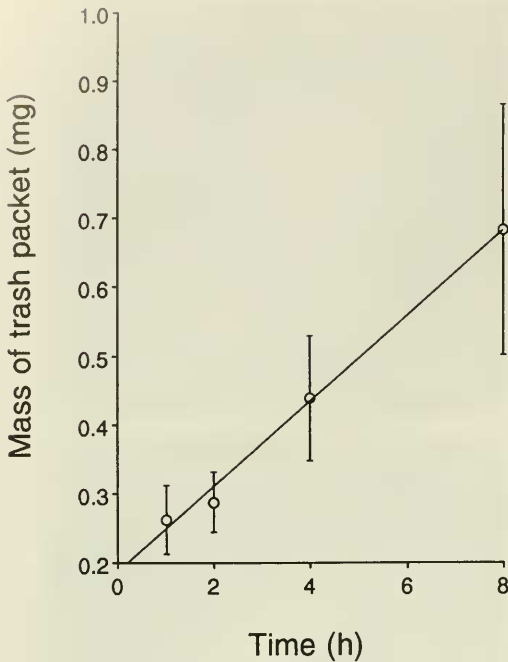


Fig. 3. Regeneration of packet by denuded larvae, expressed as mass of packet formed over time. Best fit equation is shown ($y = 0.185 + 0.062x$; $R^2 = 0.994$, $P < 0.001$).

with unstained trichomes. Front half of packet made up mostly of black trichomes. The larva evidently repaired the rear of packet with trichomes from the front, and replaced the trichomes removed from the front with ones newly acquired.

(3) The missing left half of packet was restored with newly acquired black trichomes. Only few new trichomes had been added to the right half of the packet.

(4) The entire packet had been augmented by a sparse uniform addition of new black trichomes.

It is clear from these results that the larvae are able to repair their packet, and that in so doing they do not merely fill in damaged sites with newly acquired trichomes. They are able to effect repair by repositioning existing trichomes, and they correct for any resulting inequities in trichome distribution by bringing into place newly scooped up trichomes. Thus, the larvae have not only a sense of what is missing in

a damaged packet but an elaborate mechanism for repairing the damage. Particularly remarkable is the larva's ability to move trichomes from one area of the packet to another. Casual observation reveals that they effect such relocation both by use of the jaws and by a sort of wave-like peristaltic action of the abdomen, whereby trichomes are transferred from one set of bristles to the next. Such peristaltic action was also noted at times during the package rebuilding undertaken by denuded larvae.

Trichome load of sycamore leaves.—Two procedures were adopted for determining the mass of trichomes per unit surface area of sycamore leaf underside.

One procedure involved taking a sycamore leaf, determining its surface area (by weighing a piece of paper in the shape of that leaf, and referencing this weight to that of a piece of known area of the paper), then removing the trichomes from the leaf underside (with forceps and by scraping with a scalpel) and weighing the trichomes (Fig. 1C). Removal of trichomes was judged to be 90% complete. The leaf (90.3 cm² surface area) yielded 1.2 mg trichomes, an equivalent of 13.3 μg per cm² of leaf underside.

The second procedure involved using denuded larvae to effect the trichome removal from a leaf. The leaf was exposed (underside up, with the upper side inaccessible) to two sets of 3 larvae each, operating in sequence for a combined period of 3 hours, by the end of which time the leaf underside was estimated to have lost 90% of its trichomes. The combined mass of the trash packets (which none of the larvae had completed in the time allocated) was 1.0 mg. It follows from this value, and from the area of the leaf (74.2 cm²) that the leaf had yielded 13.5 μg trichomes per cm² of underside.

Packet construction.—To obtain some measure of the rate at which the larvae construct their packets, 32 larvae were denuded and their packets weighed. They were then individually confined in plastic enclosures

Table 1. Packet regeneration of denuded larvae. See also Fig. 3. N = 8 per category, except for 8-hour category, where N = 6.

Time since larvae were denuded (hours)	New packet	
	Mass (mg)	Proportion of original packet
1	0.263 ± 0.050	0.149 ± 0.047
2	0.288 ± 0.044	0.143 ± 0.021
4	0.438 ± 0.091	0.221 ± 0.052
8	0.683 ± 0.182	0.430 ± 0.167

containing fresh sycamore leaves, and allowed to reload for 1, 2, 4, or 8 hours. Eight larvae were initially assigned to each time category, but the sample size of the 8-hour group was reduced to 6 because 2 of the larvae had affixed themselves, preparatory to pupation, during the experimental period. At the end of the assigned times, the larvae were again denuded and their packets weighed.

From the data it was possible to calculate, first of all, the average mass of the initial trash packets of the larvae (in other words, the average mass of packets of nearly full grown field-collected larvae). That mass was 2.16 ± 0.13 mg (range = 0.70–3.30 mg). In addition, a value could be obtained for each larva of the fraction of the initial packet that had been rebuilt in the time period assigned to that larva. The mean of such values, for each of the four larval categories, is given in Table 1, which also presents averages for the net mass of packet material gathered in the times allocated. A graphic representation of the latter values (Fig. 3), shows packet reconstruction to proceed at a steady rate, at least during the initial 8 hours of rebuilding.

Predation tests: reduviid.—Five nymphs of *P. laticarpus* were set up in plastic containers and individually offered equal numbers (one of each, or four of each) of packet-bearing and denuded larvae. The test chambers were checked visually at intervals, and survivorship of the larvae was recorded after 12–24 hr. Of the 12 denuded larvae that were offered, only three sur-

vived. By contrast, of the 12 shield-bearers, nine survived. Some of the packet-bearing larvae were used with more than one of the reduviids. Inspection of the cages during the experimental period had revealed some of the details of the encounters. Denuded larvae tended to be grasped the moment they were located, then promptly impaled on the reduviid's proboscis and sucked out (Fig. 4G). Packet-bearing larvae, in contrast, were held by the bug with the forelegs, repeatedly probed with the proboscis (Fig. 4F), but then often released. We suspect that release was a consequence of the reduviid's proboscis being too short to reach through the packet to the body of the larva. This interpretation is supported by an early observation in which a few larvae that were partly denuded and offered to *P. laticarpus*, were eaten.

Predation tests: ants.—The tests were set up on barren ground, about 0.5 m from the nest entrance of a *P. barbatus* colony. Tapping the ground above the nest had thrown the ants into a state of agitation and they had emerged from the nest in numbers to mount guard around the entrance. Six of 8 denuded larvae released on the ground were encountered by individual ants and carried off. They were small relative to the ants and were not always noticed by the ants when first released. In fact, it was only upon the second or third presentation that the six larvae were taken. Of 8 packet-bearing larvae that were similarly released, only one was taken, even though they were all also retested individually two or three times. The larvae were encountered repeatedly by individual ants, but they "froze" when contacted, and the ants, which in their palpations appeared to touch the packet only (Fig. 4E), were quick to abandon the assault.

DISCUSSION

While our predation data are scant, and our predation tests not nearly as satisfactory in design as we would have liked, it seems reasonable to conclude that the trichome

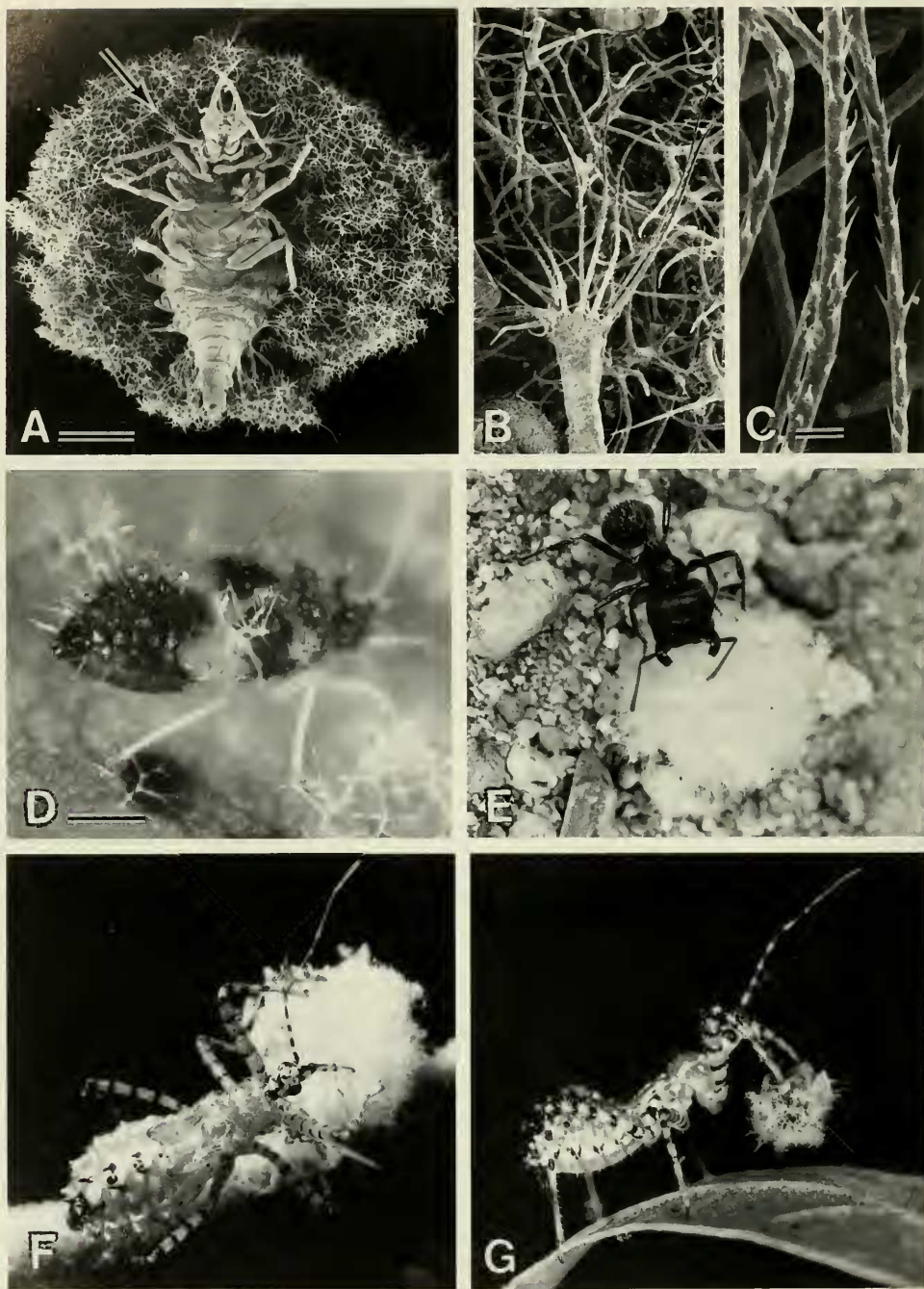


Fig. 4. A. Fully loaded larva in ventral view (scanning electronmicrograph); arrow points to one of the setose lateral protuberances that serves for retention of the packet. B. Enlarged view of a lateral protuberance. C. Detail of setae from a lateral protuberance; the barbs presumably help retain the packet. D. Nymph of the tingid *Corythucha confraterna*; note glistening droplets of secretion at tip of glandular hairs. E. An ant (*Pogonomyrmex barbatus*) inspecting a chrysidid larva. F. Reduviid nymph (*Pselliopus latispina*) probing a packet of a chrysidid larva. Most such inspections resulted in the larva being released. G. Reduviid feeding on a denuded larva. Reference bars: A = 1 mm; C = 20 μ m; D = 0.5 mm.

packet of the *C. lineaticornis* larvae is a protective structure. While ours may be the first demonstration that the trash packet of a larval chrysopid can shield against the attack of a reduviid, work with other chrysopid larvae had shown the packets to provide effective defense against other Hemiptera (New 1969) and ants (Principi 1946, Eisner et al. 1978). The strategy of carrying overhead shields among insects is, of course, not restricted to chrysopid larvae. Classic examples are provided by chrysomelid beetle larvae, including notably tortoise beetle larvae (Olmstead and Denno 1992, 1993; Gómez et al. 1999; Müller and Hilker 1999; Eisner and Eisner 2000), in which the shields are usually fabricated in part with feces. By virtue of the fecal component, such shields have the capacity to deter by chemical in addition to physical action (Gómez et al. 1999), a property that sets them apart from chrysopid packets. To our knowledge, chemical noxiousness has never been demonstrated for a chrysopid packet.

It was of some interest to find that the *C. lineaticornis* larva fed on a tingid, and specifically on the nymphs thereof. Tingid nymphs, including those of *C. confraterna* (Fig. 4D), are endowed with glandular hairs (Livingston 1978), which in some species have been shown to produce a mixture of aromatic and alicyclic acetogenins (Oliver et al. 1990), potentially deterrent to predators. *Ceraeochrysa lineaticornis* is evidently undeterred by this glandular material.

Packet construction is clearly a matter of high priority for the larva. When denuded it always made prompt efforts to rebuild the packet, and when packets were partially destroyed it always proceeded to repair the structure. Packet construction has been shown to be a matter of priority also in another chrysopid, *Chrysopa slossonae* (Banks) (Eisner et al. 1978, Milbrath et al. 1993), and given that trash packets are doubtless defensive in chrysopids generally, they are probably maintained fastidiously by all larvae that possess them. In *Cer-*

aeochrysa cincta (Schneider), for instance, a species in which the larva cloaks itself with the wax of homopteran prey, the larvae engage in packet construction immediately upon descending from the egg along the egg stalk (Mason et al. 1991).

Packet construction from sycamore trichomes is a slow process. After a period of 8 hours, nearly full grown larvae that bore packets weighing on average about 2.2 mg before being denuded, had reconstituted an equivalent of only about 40% of the original shield. It can be inferred from this that the rebuilding of an entire shield should take such larvae over a day. In fact, if one assumes reloading to proceed indefinitely at the linear rate prevailing over the first 8 hours (Fig. 3), one can calculate from the best-fit equation pertinent to that rate that it would take larvae on average nearly 32 hours to reproduce the initial packet.

The number of trichomes that go into the construction of the larval packet is substantial. A mature larva, bearing an average-sized packet of 2.2 mg, carries an amount of trichomes equivalent to what is obtainable from two sycamore leaf undersides.

Are the trichomes defensive for the sycamore itself, and does *C. lineaticornis* exemplify yet another case of utilization by an insect of defenses that evolved in the first place for protection of a plant? Sycamore trichomes can be envisioned to serve in a multiplicity of capacities. They could, for example, act to trap a layer of "dead air" directly adjacent to the leaf surface and by so doing provide for retardation of evaporative water loss from the leaves. But this does not rule out the possibility that the trichomes serve also in defense. Tightly spaced and barbed, the trichomes could well be a hindrance to many an ambulatory arthropod. The chrysopid and its tingid prey could thus be viewed as specialists that have managed to access a niche not generally open to colonization. The tingid has come to cope with the plant's defense and has become the plant's enemy. The chrysopid in contrast, is essentially the plant's

ally. Although it usurps the plant's defense, and uses the weaponry for its own purpose, it feeds on the tingid, and by so doing has become the plant's "delousing" agent. Losing a fraction of its trichomes to the chrysopid must thus be viewed as being beneficial to the sycamore.

ACKNOWLEDGMENTS

Supported in part by National Institutes of Health grant AI02908. We thank the late Robert Silberglied for identifying the reduviid and the ant, and Catherine and Maurice Tauber for countless personal favors and advice on chrysopids, and for calling attention to citations we had missed. The late Ellis MacLeod deserves thanks as well for helpful comments on our findings. This is paper no. 177 in the series "Defense Mechanisms of Arthropods"; no. 176 is Attygalle et al. 2001. Defense by foot adhesion in a chrysomelid beetle (*Hemisphaerota cyanea*): Characterization of the adhesive oil. *Zoology* 103: 1–6.

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